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Type VII collagen regulates expression of OATP1B3, promotes front-to-rear polarity and increases structural organisation in 3D spheroid cultures of RDEB tumour keratinocytes

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Abstract: Type VII collagen is the main component of anchoring fibrils, structures that are integral to basement membrane homeostasis in skin. Mutations in the gene encoding type VII collagen COL7A1 cause recessive dystrophic epidermolysis bullosa (RDEB) an inherited skin blistering condition complicated by frequent aggressive cutaneous squamous cell carcinoma (cSCC). OATP1B3, which is encoded by the gene SLCO1B3, is a member of the OATP (organic anion transporting polypeptide) superfamily responsible for transporting a wide range of endogenous and xenobiotic compounds. OATP1B3 expression is limited to the liver in healthy tissues, but is frequently detected in multiple cancer types and is reported to be associated with differing clinical outcome. The mechanism and functional significance of tumour-specific expression of OATP1B3 has yet to be determined. Here, we identify SLCO1B3 expression in tumour keratinocytes isolated from RDEB and UV-induced cSCC and demonstrate that SLCO1B3 expression and promoter activity are modulated by type VII collagen. We show that reduction of SLCO1B3 expression upon expression of full-length type VII collagen in RDEB cSCC coincides with acquisition of front-to-rear polarity and increased organisation of 3D spheroid cultures. In addition, we show that type VII collagen positively regulates the abundance of markers implicated in cellular polarity, namely ELMO2, PAR3, E-cadherin, B-catenin, ITGA6 and Ln332.

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Type VII collagen regulates tumour expression of organic anion transporting polypeptide OATP1B3, promotes front to rear polarity and increases structural organisation in 3D spheroid cultures of recessive dystrophic epidermolysis bullosa tumour keratinocytes

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Running head: Collagen VII promotes tumour cell polarity

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SUMMARY

Type VII collagen is the main component of anchoring fibrils, structures integral to basement membrane homeostasis in skin. Mutations in the gene encoding type VII collagen, *COL7A1*, cause recessive dystrophic epidermolysis bullosa (RDEB) an inherited skin blistering condition complicated by frequent aggressive cutaneous squamous cell carcinoma (cSCC). OATP1B3, encoded by the gene *SLCO1B3*, is a member of the OATP (organic anion transporting polypeptide) superfamily responsible for transporting a wide range of endogenous and xenobiotic compounds. OATP1B3 expression is limited to the liver in healthy tissues but is frequently detected in multiple cancer types and is reported to be associated with differing clinical outcome. The mechanism and functional significance of tumour specific OATP1B3 expression has yet to be determined. Here, we identify *SLCO1B3* (encoding OATP1B3) expression in tumour keratinocytes isolated from RDEB and UV induced cSCC and demonstrate that *SLCO1B3* expression and promoter activity are modulated by type VII collagen. We demonstrate that reduction of *SLCO1B3* expression upon full length type VII collagen expression in RDEB cSCC coincides with acquisition of front to rear polarity and increased organisation of 3D spheroid cultures. In addition we show that type VII collagen positively regulates the abundance of markers implicated in cellular polarity, namely ELMO2, PAR3, E-cadherin, B-catenin, ITGA6 and Ln332.

INTRODUCTION

Non-melanoma skin cancer (NMSC) incidence is highest among all malignancies in Caucasian populations (Toms, 2004; CancerResearchUK, 2012). Approximately 80% of NMSC comprise basal cell carcinoma (BCC) and 20% cutaneous squamous cell carcinoma (cSCC) (Alam and Ratner, 2001; Yanofsky et al., 2011). BCC is usually a benign disease and is rarely responsible for skin cancer related mortality whereas cSCC can develop into metastatic disease and contributes to around 1 in 4 skin cancer deaths in the UK (ISD Scotland; <http://www.isdscotland.org/isd/183.html>). The main risk factor for cSCC in the general population is UV radiation (Fears and Scotto, 1983; Preston and Stern, 1992) but other factors such as HPV infection (McGregor and Proby, 1996; Harwood and Proby, 2002) and immunosuppression in organ transplant patients (Rowe et al., 1992; Veness et al., 1999) have been documented. Inherited skin disease can also predispose to developing cSCC (Ng et al., 2011); in particular, individuals diagnosed with recessive dystrophic epidermolysis bullosa (RDEB) (Reed et al., 1975; Fine et al., 2009). Patients with this disease are affected by deleterious mutations in *COL7A1* leading to either defective or absent type VII collagen (Christiano et al., 1993). Type VII collagen is the main component of anchoring fibrils, structures which associate with the basement membrane and are essential for the proper attachment of the dermis to the epidermis (Sakai et al., 1986; Burgeson, 1993). Type VII collagen interacts with proteins localised close to the basement membrane; laminin 332 (Ln332) and type IV collagen (Chen et al., 1997; Rousselle et al., 1997), as well as banded collagen I containing fibrils localised in the dermis (Villone et al., 2008) providing strong adhesive connection between these two compartments. Over 90% of RDEB patients develop metastatic cSCC by age 55, a figure significantly higher than the general population where metastatic disease is relatively rare (Weinstock et al., 1991; Miller and Weinstock, 1994; Fine et al., 2009). The underlying molecular mechanism driving predisposition to develop aggressive cSCC in RDEB is not fully understood (South and O'Toole, 2010) although recent data from our laboratory shows that tumour microenvironment, in particular the composition of extracellular matrix in RDEB is tumour promoting (Ng et al., 2012). Similarly, despite an alarming rise in cSCC incidence in the general population and a relatively low 5-year survival in patients presenting with regional metastasis (Rowe et al., 1992; Czarnecki et al., 1994), little is understood about those tumours with poor prognosis.

We previously identified very few differentially expressed genes (DEGs) using microarray to compare RDEB with UV induced cSCC in a relatively small number of keratinocyte cultures (n=9) (Watt et al., 2011). We further investigate those 18 DEGs and identify *SLCO1B3*, a gene which encodes the liver specific organic anion transporter polypeptide OATP1B3, as being up regulated in both UV induced and RDEB cSCC when compared with normal skin. Transporter-genes are biologically significant for cell homeostasis and can regulate cellular mechanisms such as metabolism, cell death and motility (Olk et al., 2009; Klaassen and Aleksunes, 2010). OATP1B3 is normally expressed in the liver and is involved in the transport of bile salts, glutathione and other organic anions (Smith et al., 2005a; Hagenbuch and Gui, 2008). In agreement with our data, OATP1B3/*SLCO1B3* is frequently up-regulated in solid tumours including breast and colon (Lockhart et al., 2008; Maeda et al., 2010; Pressler et al., 2011) and it has been suggested that OATP1B3 has therapeutic potential owing to its ability to transport a broad range of drugs including methotrexate, paclitaxel, docetaxel, bromsulphalein (BSP) and pitavastatin (Smith et al., 2005b; Hagenbuch and Gui, 2008). OATP1B3 expression has been reported to associate with clinical outcome in a number of studies (Hamada et al., 2008; Lockhart et al., 2008) yet little data exist on the mechanism of expression or functional consequence. Lee and colleagues report that OATP1B3 promotes apoptotic resistance in tumour cells (Lee et al., 2009) while very recently hypoxia has been shown to positively regulate OATP1B3 expression (Ramachandran et al., 2013)

Here, we investigate the relationship between type VII collagen and tumour expression of *SLCO1B3* and demonstrate that type VII collagen, Ln332 and ELMO2 regulate tumour expression of *SLCO1B3*. We also go on to show that expression of type VII collagen in RDEB cSCC keratinocytes promotes front to rear polarity on uncoated plastic and increases organisation of 3D spheroid cultures. These structural changes in response to type VII collagen expression coincide with an increase in the abundance of markers implicated in cellular polarity, namely ELMO2, PAR3, E-cadherin, B-catenin, ITGA6 and Ln332.

RESULTS

***SLCO1B3* is expressed in cSCC in vitro and in vivo**

We previously identified only 18 genes to be significantly differentially expressed between cultured RDEB cSCC keratinocytes and UV induced cSCC keratinocytes using gene expression arrays demonstrating these two tumour groups are not significantly different in this assay (Watt et al., 2011). Initial follow-up of these 18 genes comparing 3 RDEB cSCC and 3 UV cSCC cultures using sybr®green qPCR revealed 12 genes showed the same trend as array data but only 5 were significantly dysregulated: *SLCO1B3*, *DUSP23*, *TJAP1*, *SNRPN* and *SNURF* (data not shown). From these five genes, *SLCO1B3* showed a consistent up-regulation in RDEB cSCC keratinocytes in culture compared with normal primary keratinocytes and the majority of UV induced cSCC keratinocytes, with the exception of SCCT8 (Fig. 1A). However this experiment also identified a significant increase in expression in the majority of UV induced cSCC keratinocytes when compared with normal keratinocytes. Six of eight cultured cSCC cell lines showed a greater level of expression compared with the hepatocellular carcinoma cell line HepG2 (Fig. 1A).

Q-PCR using reverse transcribed mRNA obtained from patient tissues showed high *SLCO1B3* expression in RDEB cSCC, and to a lesser extent UV induced cSCC and RDEB non-SCC skin compared with normal skin, with the highest levels evident in RDEB cSCC tissue (Fig. 1B).

Endogenous OATP1B3 expression is detectable in cSCC keratinocytes but only localises to the membrane in vivo

OATP1B3 expression was readily detected in cSCC keratinocytes but not in normal primary keratinocytes in culture (Fig. 2A). Plasma membrane bound OATP1B3 was however not observed in cultured cSCC keratinocytes using indirect immunofluorescence unless over-expressed via retroviral transduction (Fig. 2B). Membrane bound protein was detected in organotypic 3D cultures (data not shown) and xenograft tumours (Fig. 2B right panels).

Tissue sections obtained from RDEB cSCC (n=7), RDEB skin (n=3) and UV induced cSCC (n=6) patients along with normal human liver (positive control) and normal human skin

(negative control) were assessed for OATP1B3 expression. 6/7 RDEB cSCC and 5/6 cSCC samples showed heterogeneous plasma membrane bound protein expression along with nuclear localisation in 3/7 RDEB cSCC and 2/6 cSCC tissues (Fig. 3A upper panels and Fig. 3B). 1/3 RDEB skin samples showed membrane bound protein (Fig. 3A lower panels). Co-localisation with plakoglobin confirmed membrane localisation although higher levels of OATP1B3 were readily detected in regions of cSCC tissue where membrane bound plakoglobin was absent (Fig. 3B).

***SLCO1B3* expression is regulated by type VII collagen**

We noted that when wild-type *COL7A1* was expressed in RDEB cSCC keratinocytes (SCCRDEB-C7 cells) the level of *SLCO1B3* expression was reduced compared with empty vector control cells (SCCRDEB-EV cells) ($p=0.004$, Fig. 4A). This observation was confirmed using separate clonal populations of transduced cells ruling out any insertion effects (Fig. 4A-C). To determine whether type VII collagen had a direct effect on promoter activity of *SLCO1B3* we cloned the *SLCO1B3* promoter, previously described in Jung et al., 2002 (Jung et al., 2002) and transfected a *SLCO1B3* promoter-driven luciferase construct into either SCCRDEB3-C7 or SCCRDEB3-EV cells. *SLCO1B3* promoter activity was significantly ($p=0.02$) reduced in collagen VII transduced SCCRDEB3 keratinocytes compared to control cells (Fig. 4D).

Further, *SLCO1B3* expression was significantly reduced in two separate patient derived parental populations of RDEB SCC cells, SCCRDEB3 and SCCRDEB2, when cultured on matrix derived from SCCRDEB3-C7 cells compared to matrix derived from SCCRDEB3-EV vector control cells (Fig. 4E).

We then used siRNA mediated depletion of *COL7A1* to show that reduction of recombinant type VII collagen increases *SLCO1B3* expression in a clonal population of SCCRDEB3-C7 cells (Fig. 5A-B). We note that in this experiment the NT control level of *SLCO1B3* was greater in SCCRDEB3-EV cells than SCCRDEB3-C7 cells as expected (1.5 fold) yet the impact of siRNA mediated depletion of *COL7A1* resulted in a greater increase in *SLCO1B3* levels than seen in the EV population (Fig. 5B). We attribute this observation to the clonal nature of the two cell populations. To confirm the observation that siRNA depletion of *COL7A1* increased *SLCO1B3* expression in non-clonal populations we next used siRNA

depletion of *COL7A1* in SCCRDEB4-C7 cells (Fig. 5C) and the UV induced, nonRDEB cSCC keratinocyte population, SCCT8 (Fig. 5D) providing a direct link to type VII collagen and *SLCO1B3* tumour keratinocyte expression. The same effect was also observed in normal primary keratinocytes (NHK) following type VII collagen knock down, although the overall level of expression was significantly lower than in tumour cells (supplementary Fig. 1).

Expression of the polarity related protein ELMO2 is regulated by type VII collagen

OATP1B3 is a transmembrane transporter and is expressed in the basolateral component of the plasma membrane. Type VII collagen is a basement membrane protein that directly binds to Ln332 which itself binds with integrin receptors. It has been previously reported that expression of cell surface proteins can be regulated by interactions with basement membrane components that influence cellular polarity (Li et al., 2003; Daley et al., 2012). We therefore postulated that although OATP1B3 was not observed to be asymmetrically localised in tumour keratinocytes adjacent to extracellular matrix (Fig.3) absence of type VII collagen might regulate its expression by altering cellular polarity.

Other than basement membrane associated proteins in basal keratinocytes or tight junction components in upper stratified keratinocytes, asymmetrically distributed apical basal polarity markers have yet to be defined in the epidermis (Niessen et al., 2012). Better described is the redistribution of proteins involved in the assembly and disassembly of focal adhesions during the acquisition of front to rear polarity in cultured single cells (Hamill et al., 2009; Ho and Dagnino, 2012b). Front to rear polarity in cultured primary murine keratinocytes in response to Ln332 is mediated in part by a focal adhesion complex containing the relatively novel protein engulfment and motility 2, or ELMO2 (Ho et al., 2009; Ho and Dagnino, 2012b). We therefore investigated whether a relationship existed between ELMO2, type VII collagen and OATP1B3.

Overexpression of type VII collagen resulted in up regulation of *ELMO2* expression in SCCRDEB3-C7 cells (Fig. 6A). Concurrently, siRNA mediated depletion of *COL7A1* in four independent tumor cell populations resulted in a reduction in *ELMO2* expression; SCCRDEB3-C7 and SCCRDEB4-C7 cells as well as cSCC cells that endogenously express type VII collagen; SCCT8 and SCCT1 (Fig. 6B). ELMO2 protein levels corresponded with reduction in transcript (Fig. 6C-D). Further, *ELMO2* expression increased in both

SCCRDEB3 and SCCRDEB2 keratinocytes that were cultured on matrix derived from SCCRDEB3-C7 cells (Fig. 6E-F). These data demonstrate that type VII collagen positively regulates *ELMO2* expression in cSCC keratinocytes.

We next investigated the link between *ELMO2* and *SLCO1B3* expression and showed that overexpression of *ELMO2* resulted in a reduction in *SLCO1B3* mRNA levels in SCCT8 and SCCRDEB2 (Fig. 7A-B), whereas a reduction in *ELMO2* mRNA resulted in an increase in *SLCO1B3* expression in SCCT8 (Fig. 7C-D) and non-SCC RDEB keratinocytes (supplementary Fig. 2A-C), with a significant increase in *SLCO1B3* observed in normal primary keratinocytes after *ELMO2* reduction (supplementary Fig. 2D-F). Thereby, demonstrating that like *COL7A1*, *ELMO2* can also negatively regulates *SLCO1B3*.

Type VII collagen can regulate *SLCO1B3* independent of *ELMO2*

To confirm whether expression of *SLCO1B3* in cSCC was dependent on expression of either type VII collagen or *ELMO2* alone, we investigated *SLCO1B3* expression following *ELMO2* knock down in type VII collagen overexpressing RDEB cSCC cells (RDEBSCC3-C7) and control cells (RDEBSCC3-EV). *SLCO1B3* expression significantly increased in RDEBSCC3-EV cells following *ELMO2* knockdown demonstrating *ELMO2* can regulate *SLCO1B3* in the absence of type VII collagen (supplementary Fig. 3A-B). In RDEBSCC3-C7 cells no significant difference in *SLCO1B3* expression was observed following *ELMO2* knock down (supplementary Fig. 3A-B), showing that *ELMO2* depletion had no effect on *SLCO1B3* expression. In addition we show that *ELMO2* does not regulate type VII collagen, as observed in SCCT8 (supplementary Fig. 3C-E) or normal primary keratinocytes (supplementary Fig. 2A-C) following *ELMO2* knock down. We also investigated Ln332, a binding partner of type VII collagen and also known to regulate polarity in epithelial cells (Kligys et al., 2007). In RDEB cSCC keratinocytes Ln332 siRNA mediated knock down resulted in an increase in *SLCO1B3* message levels (Fig. 7E-F). This effect was independent of *ELMO2* expression (supplementary Fig. 3F). Collectively these data show that *SLCO1B3* is regulated independently by proteins with known roles in epidermal polarity (*ELMO2* and Ln332) and that *SLCO1B3* expression is controlled through a single mechanism governed by either of these proteins or type VII collagen alone.

Type VII collagen regulates front to rear keratinocyte polarity in 2D culture and regulates structural organisation in 3D spheroid culture

Ln332 and ELMO2 have previously been shown to play a role in epidermal polarity (Li et al., 2003; Ho and Dagnino, 2012b). To investigate whether type VII collagen ~~and~~ could also regulate keratinocyte front to rear polarity we seeded RDEBSCC C7 cells at low density and assessed lamellipodia formation at 5 hours as described (Hamill et al., 2009) (Fig. 8A). Type VII collagen expression marginally increased the number of attached cells that spread onto uncoated plastic (Fig. 8B) but strikingly increased the number of lamellipodia observed (Fig. 8C). Cells displaying front to rear polarity were frequently observed in RDEBSCC C7 cells but were completely absent in vector control cells after 5 hours (Fig. 8A).

The same experiment was repeated in RDEBSCC3 cells following ELMO2 transient overexpression to confirm the role of ELMO2 in RDEB cSCC polarity. ELMO2 overexpressing cells (ELMO2) showed a significant increase in the number of polarised cells compared to control cells (EV) (supplementary Fig. 4).

To investigate whether type VII collagen can influence structural organisation, 3D spheroid cultures were established with SCCRDEB3-C7 cells and compared with the empty vector controls, SCCRDEB3-EV. SCCRDEB3-C7 spheroids showed increased structural organisation determined by morphology (Fig. 8D) and overall increased levels of proteins previously associated with cellular polarity, namely PAR3, ELMO2, integrin alpha 6 and Ln332 when compared with SCCRDEB3-EV spheroids (Fig. 8D-E). Next we assessed the effect of stromal derived type VII collagen on RDEB SCC cells. To do this we co-cultured parental SCCRDEB3 cells with fibroblasts either expressing recombinant type VII collagen or vector control in a spheroid assay over 7 days. After this time we observed that mesenchyme delivery of type VII collagen had a more profound effect on RDEBSCC keratinocytes in spheroid culture than RDEBSCC keratinocytes cultured alone in the presence or absence of type VII collagen. Spheroid cultures of RDEBSCC cells with fibroblast delivered type VII collagen exhibited more distinct separation of cellular populations and a clear increase in distribution to the membrane of beta-catenin and e-cadherin (Fig. 8F). Western blot analyses showed no overall changes in e-cadherin and beta-catenin protein levels (Fig. 8G) suggesting that the differences observed in solvent fixed embedded spheroids (Fig. 8F) are a result of membrane localisation.

DISCUSSION

cSCC is the most common cause of NMSC mortality and represents a significant clinical complication in high risk groups, particularly patients with RDEB. UV exposure resulting in DNA damage is a well-established driver of cSCC (Armstrong and Kricker, 2001). On the contrary, RDEB associated cSCC disease aggression is regarded as UV independent and although tumour initiation is still not fully understood (Fine et al., 2009) recent work from our laboratory demonstrates the role of microenvironment in RDEB tumour progression (Ng et al., 2012). Here our study began by interrogating a small number of changes identified through expression array analysis comparing UV with RDEB cSCC keratinocytes in culture. In agreement with multiple previous studies little differences were confirmed between these two tumour types (Arbiser et al., 2004; Mallipeddi et al., 2004). In fact our primary target, *SLCO1B3* is aberrantly expressed in a panel of cSCC from both UV induced and RDEB induced cSCC cell lines and tissue (Fig. 1), again in agreement with previous studies identifying *SLCO1B3* expression in cancer (Maeda et al., 2010; Pressler et al., 2011; Svoboda et al., 2011). However, by pursuing the molecule responsible for RDEB we show that type VII collagen regulates the expression of *SLCO1B3* in both RDEB cSCC and UV induced cSCC (Fig. 3). Further to this we demonstrate that front to rear polarity and organisation of 3D spheroid culture coincides with changes in expression of *SLCO1B3* in cSCC keratinocytes when type VII collagen levels are altered (Fig. 8).

Polarity is essential for the maintenance of tissue homeostasis and can either be defined as the distribution of apical and basal membrane domains within the cell or the positioning of a cell within the tissue itself, referred to as planar cell polarity (PCP). Directional transport, asymmetric stem cell division, oriented cell division and sensing signals from neighbouring microenvironment are a few examples of cellular processes that require the maintenance of cell and tissue polarity (Lechler and Fuchs, 2005; Martin-Belmonte and Mostov, 2008; Poulson and Lechler, 2010; Martin-Belmonte and Perez-Moreno, 2012). Previously, three main protein complexes have been implicated in maintenance of polarity in simple epithelia, Par6-Par3-aPKC (Munro, 2006; Goldstein and Macara, 2007), Crumbs-Stardust-PATJ complex and Scribble-Lethal giant larvae (Lgl)-Discs large (Dlg) complex (Martin-Belmonte and Mostov, 2008; Martin-Belmonte et al., 2008). However, the role of these protein complexes in maintenance of stratified epithelia polarity is not clear (Muroyama and Lechler, 2012; Niessen et al., 2012) and recent work has shown that the engulfment and cell motility

protein, ELMO2, positively regulates front to rear keratinocyte polarity by forming complexes with ILK in the presence of EGF (Ho et al., 2009; Ho and Dagnino, 2012a; Ho and Dagnino, 2012b). The data presented here identify for the first time a link between ELMO2 and type VII collagen demonstrating that, like ELMO2, type VII collagen can regulate front to rear polarity (Fig. 8A). We go on to demonstrate that like type VII collagen, ELMO2 negatively regulates *SLCO1B3* (Fig. 7 and supplementary Fig. 2) and this effect is independent of the regulation of ELMO2 by type VII collagen (supplementary Fig. 3). We also show that Ln332 regulates *SLCO1B3* independently of ELMO2 (Fig. 7E-F). Together these data suggests that *SLCO1B3* expression is not modulated by a common mechanism underlying an interaction between type VII collagen and ELMO2 but instead suggests that *SLCO1B3* expression is modulated by cellular polarity.

Type VII collagen and Ln332 are secreted proteins important for epidermal keratinocyte attachment (Ogawa et al., 2007). Type VII collagen binds Ln332, a key component of hemidesmosomes and focal adhesions (Chen et al., 1999). Numerous components of these adhesion complexes have been implicated in regulating epithelial polarity (Cox et al., 2001; Martin-Belmonte and Perez-Moreno, 2012) and indeed ELMO2 is shown to regulate front-to-rear polarity in the presence of Ln332 (Ho et al., 2009; Ho and Dagnino, 2012a). Similarly, integrin $\alpha 6 \beta 4$ regulates keratinocyte attachment via Ln332 and has a well-established role in directional migration (Jones et al., 1991; Dowling et al., 1996) since integrin activation of focal adhesion kinases is required for leading edge formation and directional movement (Lim et al., 2010). Integrin $\alpha 6 \beta 4$ also interacts with BPAG1e, promoting keratinocyte polarity through modulation of RAC1 (Hamill et al., 2009). We have shown that in the absence of type VII collagen ITGA6 and Ln332 expression is significantly reduced in 3D spheroid cultures (Fig. 8), suggesting a common mechanism, although further work is necessary to confirm this.

E-cadherin and beta-catenin have described roles in numerous polarity pathways; APC (den Elzen et al., 2009), SCRIB (Navarro et al., 2005), PAR3 (Le Borgne et al., 2002), and Hippo (Kim et al., 2011); all of which are characterised by a down-regulation in cancer progression (Muroyama and Lechler, 2012; Niessen et al., 2012). Our data showing type VII collagen regulates the expression of membrane bound e-cadherin in 3D spheroid culture is in agreement with a previous study of siRNA depletion of *COL7A1* cSCC showing reduced e-cadherin in 3D organotypic culture (Martins et al., 2009). Further work will be necessary to

determine the precise role that individual complexes play in type VII collagen mediated polarity and organisation. Toward this end we show here a reduction of PAR3 in RDEB cSCC spheroid culture and it is interesting to note that altered PAR3 distribution has been observed as a result of reduced e-cadherin expression (Le Borgne et al., 2002) and that deletion of PAR3 in murine epidermis does not alter normal skin development or function but does favour keratoacanthoma formation. Consequently, PAR3 wildtype mice show increased SCC formation on a background of RAS mutations, suggesting a dual role of PAR3 in skin carcinogenesis (Iden et al., 2012).

Reorganisation of polarity can have a profound effect on proteins located at the cell surface. OATP1B3 is a transmembrane transporter that localises in the basolateral domain of the plasma membrane in normal liver and the data presented here strongly support a change in polarity as an explanation for *SLCO1B3* expression on the background of altered tumour cell behaviour. In addition to the identification that type VII collagen regulates OATP1B3 expression we confirm loss of plasma membrane bound OATP1B3 expression in 2D cultured cells, a phenomenon also documented in primary hepatocytes (Ulvestad et al., 2011). Two recent studies have identified an N-terminal truncated isoform of OATP1B3, OATP1B3 V1, (lacking the first 28 amino acids) as being the predominant OATP1B3 expressed in colon, lung and pancreatic cancer (Nagai et al., 2012; Thakkar et al., 2013). Thakkar and colleagues demonstrate that OATP1B3 V1 localises predominantly to the cytoplasm in culture (Thakkar et al., 2013). Our data show that membrane localised OATP1B3 is absent in cultured SCCT8 cells but readily detected when these cells are grown in vivo (Fig. 2C). Therefore a more detailed analysis of OATP1B3 isoforms expressed in cSCC under these varied conditions is warranted. Since it has been suggested that OATP substrates and inhibitors can be used either to enable uptake of xenobiotics for the specific targeting of cancer cells or by inhibiting OATP mediated transport of hormones to prevent resistance to existing therapies (Maeda et al., 2010; Svoboda et al., 2011; Obaidat et al., 2012) it will be of interest to understand the relationship between transport and isoform expression.

In summary we show that tumour expression of *SLCO1B3* in cSCC can be regulated by type VII collagen and ELMO2 and that expression of type VII collagen in RDEB SCC keratinocytes increases front to rear polarity and structural organisation in spheroid culture coinciding with dramatic reorganisation of β -catenin and e-cadherin protein localisation.

MATERIALS AND METHODS

All human samples were collected after informed, written consent and in accordance with Helsinki guidelines. All animals were used in accordance with UK Home Office regulations.

Keratinocyte culture and xenograft tumour material

Keratinocyte isolation, organotypic culture and xenograft tumour growth were achieved as previously described (Nystrom et al., 2005; Watt et al., 2011)

Sybr®Green qPCR validation

cDNA was prepared from RNA isolated using RNA-Bee (AMS Biotechnology, Abingdon-on-Thames, UK) and the QuantiTect reverse transcription kit (Qiagen, Manchester, UK). The 2X Sybr®Green master mix (Qiagen, Manchester, UK) along with the *SLCO1B3* forward (5'-GTCCAGTCATTGGCTTTGCA-3') and reverse (5'-CAACCCAACGAGAGTCCTTAGG-3') primers, *ELMO2* forward (5'-GGGACATGGTTTCAATCACC-3') and reverse (5'-TGGAGGTGTGAGATGAGCTG-3') and previously published *COL7A1* primers (Wang et al., 2006) were used to quantify mRNA levels. *GAPDH* primers (Forward 5'-TTCCGGGAAACTGTGGCGTGA-3' and reverse 5'-ACGGAAGGCCATGCCAG-3') and previously published *ACTB* primers (Ehrenfeld et al., 2011) were used to normalise expression data. Raw data was analysed with the Rotor-Gene Q qPCR software (Qiagen Manchester, UK) using the $\Delta\Delta CT$ method.

Retroviral transduction

The open reading frame of *SLCO1B3* and *COL7A1* were cloned from an IMAGE cDNA clone (Open Biosystems, Thermo Scientific, Huntsville, AL) or normal keratinocyte cDNA respectively and inserted into the pBabe-puro vector using standard molecular biology techniques. RDEB cSCC keratinocytes were retrovirally transduced using the Phoenix

retroviral packaging system (Kinsella and Nolan, 1996). All transduced cells were selected using 2 µg/ml puromycin. SCCRDEB3 cells were then clonally selected to generate a homogenous population expressing a high level of type VII collagen. SCCRDEB4 were also transduced as described but without clonal selection.

Immunoblotting and Immunofluorescence

Protein lysates from 2 days post confluent cells were extracted using the radioimmunoprecipitation assay (RIPA) buffer and quantified using the bicinchoninic acid (BCA) assay (ThermoFisher Scientific, Waltham, USA). 4-15% Mini-PROTEAN TGX gels (Bio-Rad, Hertfordshire, UK) were used for electrophoresis.

Formalin fixed paraffin embedded (FFPE), fresh frozen tissue sections and cSCC primary keratinocytes were used. Briefly, FFPE sections were rehydrated using 100%, 95% and 70% ethanol and then treated with 1% citric acid buffer (pH 6) at 100°C. FFPE/fresh frozen sections/ keratinocytes cultured on coverslips were stained with the primary antibody for 1 hour followed by a 40 minute treatment with the secondary antibody and counterstained with DAPI (Invitrogen, Paisley, UK). Slides were imaged using the Zeiss Axioskop 2 fluorescent microscope (Carl Zeiss Microscopy, LLC, United States) and analysed using the Axiovision software or the Leica TCS SP5 confocal microscope and the Leica Application Suite Advanced fluorescence Lite software (Leica Microsystems, Wetzlar, Germany).

Antibodies

The following antibodies were used for immunofluorescence and westernblot analysis. Primary antibodies used: OATP1B3: H-52 (0.4 µg/ml, Santa Cruz, biotechnology, Dallas, Texas, USA), type VII collagen: 234192, (Calbiochem, Darmstadt, Germany), ELMO2: either the E-16 clone (0.8 µg/ml, Santa Cruz, biotechnology, Dallas, Texas, USA) or HPA018811 (0.4 µg/ml, Sigma- Aldrich, Dorset, UK), Integrin alpha 6: 3750 (Cell Signalling, Danvers, USA), e-cadherin: (2.5 µg/ml, 610101, BD Transduction Laboratories™, San Jose, CA, USA), Laminin 332: γ chain MAB19562, (1 µg/ml, Calbiochem, Darmstadt, Germany), β-catenin: SC-7199 (4 µg/ml, Santa Cruz, biotechnology, Dallas, Texas, USA), PAR3: 07-330 (0.4 µg/ml, Calbiochem, Darmstadt, Germany), Actin:

SC-58625 (0.2 µg/ml, Santa Cruz, biotechnology, Dallas, Texas, USA) and Plakoglobin: 61005, (2.5 µg/ml, Progen Biotechnik, Heidelberg, Germany). Secondary antibodies used: Alexafluor 594® rabbit (2.5 µg/ml, Invitrogen, Paisley, UK) or Alexafluor 488® mouse (2.5 µg/ml, Invitrogen, Paisley, UK)

Luciferase gene reporter assay

A luciferase gene reporter construct was created by inserting the *SLCO1B3* promoter (1Kb upstream of the 5'UTR region of the gene: 11375105 - 11376214 of accession number: NW_001838052.1) (Jung et al., 2002) into the pGL3 basic vector using standard molecular biology techniques. SCCRDEB3 cells transduced with *COL7A1* ORF or empty vector control were transfected with the *SLCO1B3* promoter construct using Lipofectamine 2000 (Invitrogen, Paisley, UK) according to the manufacturer's recommendations. Cells were lysed and harvested after 18 hours and luciferase activity was recorded using the Dual-Luciferase® reporter assay system (Promega, Madison, USA) and a Modulus II plate reader (Promega, Madison, USA). Data was normalised for transfection efficiency using renilla and empty vector control cells were used as a calibrator.

SiRNA knock down of *COL7A1*, *LAMC2* and *ELMO2*

Three siRNA constructs against *COL7A1* (SASI_Hs01_00155170/71/73, Sigma-Aldrich, Dorset, UK), *LAMC2* (SASI_Hs01_00136952/53/54, Sigma-Aldrich, Dorset, UK), *ELMO2* (SASI_Hs01_00078453/54/55, Sigma-Aldrich, Dorset, UK) and a custom designed non-targeting control (sense: GCUGCUAGACGUGUUAUGA[dT][dT], antisense: UCAUAACACGUCUAGCAGC[dT][dT], Sigma-Aldrich, Dorset, UK) were transfected using Lipofectamine 2000 (Invitrogen, Paisley, UK). RNA was extracted 24, 48 and 72 hours post transfection and analysed for *COL7A1*, *SLCO1B3*, *LAMC2* and *ELMO2* expression using Sybr®Green qPCR.

ELMO2 overexpression

An *ELMO2* cDNA construct (MHS1010-57517, Thermo Scientific, Waltham, USA) and the empty vector control was transfected into cSCC keratinocytes using Lipofectamine 2000 (Invitrogen, Paisley, UK). RNA and protein was harvested 17 hours and 24 hours post transfection and assessed for *ELMO2* overexpression using Sybr®Green qPCR.

Cell derived matrix

SCCRDEB3-C7 and SCCRDEB3-EV cells were cultured in a 6 well plate until 2 days post confluent. Cells were washed with cold PBS and lysed with 1ml 0.5% sodium deoxycholate for 10 minutes on ice. Lysed cells were removed and wells were washed with dH₂O and air dried. SCCRDEB2 and SCCRDEB3 parental cells were seeded on either collagen VII containing matrix or empty vector control matrix for 3 days before RNA and protein samples were harvested.

Cell plating lamellipodia scoring

500,000 SCCRDEB3-C7 and SCCRDEB-EV cells were seeded in a 10 cm dish. Cells were imaged 5 hrs post seeding. Twenty frames were scored blind for each cell type and data was analysed from three independent experiments. Clear cellular protrusions were counted for lamellipodia scoring and difference in cell diameter was observed in order to accurately score for cell spreading. Leica TCS SP5 confocal microscope was used to image cells after staining with β -actin and analysed using the Leica Application Suite Advanced fluorescence Lite software (Leica Microsystems, Wetzlar, Germany).

Spheroid mono-culture

SCCRDEB3 type VII collagen overexpressing (SCCRDEB3-C7) and the empty vector cells (SCCRDEB3-EV) cells were cultured for 72 hours in a hanging drop non-adherent culture. Briefly, 6000 cells were seeded on the lid of a 10 cm culture dish and allowed to form spheroids. Each spheroid was washed with PBS and harvested using a syringe and placed in 4% paraformaldehyde overnight. Spheroids were washed with PBS next day and placed at

4°C and embedded in paraffin. Alternatively, spheroids were washed with PBS and directly frozen in OCT. Spheroids were serially sectioned for immunofluorescence.

Spheroid co-culture

Fibroblasts were transduced with *COL7A1* or empty vector controls. 3,000 fibroblasts were seeded in 20 µL media on a sterile 10 cm cell culture dish lid and incubated for four days at 37°C. On day four, 10 µL of culture media was removed from each droplet. 6,000 keratinocytes were seeded in 10 µL of media onto each droplet of previously formed fibroblasts spheroids and cultured for three days. Spheroids were washed with PBS and embedded in OCT for serial sectioning and staining. In parallel and to visualise the separate populations, cells were labelled with PKH67 green fluorescent cell linker kit (fibroblasts) or PKH26 red fluorescent cell linker kit (keratinocytes) according to the manufacturer's protocol (Sigma-Aldrich, St Louis, USA). Labelled spheroids were visualised using the Carl Zeiss Axiovert25 CFL microscope (Carl Zeiss Microscopy, LLC, United States).

Statistical analysis

Student's two tailed T-test was used to determine statistical significance of luciferase gene reporter assays and *COL7A1* siRNA knockdown data.

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REFERENCES

- Alam, M. and Ratner, D.** (2001). Cutaneous squamous-cell carcinoma. *N Engl J Med* **344**, 975-983.
- Arbiser, J. L., Fan, C. Y., Su, X., Van Emburgh, B. O., Cerimele, F., Miller, M. S., Harvell, J. and Marinkovich, M. P.** (2004). Involvement of p53 and p16 tumor suppressor genes in recessive dystrophic epidermolysis bullosa-associated squamous cell carcinoma. *J Invest Dermatol* **123**, 788-790.
- Armstrong, B. K. and Krickler, A.** (2001). The epidemiology of UV induced skin cancer. *J Photochem Photobiol B* **63**, 8-18.
- Burgeson, R. E.** (1993). Type VII collagen, anchoring fibrils, and epidermolysis bullosa. *J Invest Dermatol* **101**, 252-255.
- Caddy, J., Wilanowski, T., Darido, C., Dworkin, S., Ting, S. B., Zhao, Q., Rank, G., Auden, A., Srivastava, S., Papenfuss, T. A. et al.** (2010). Epidermal wound repair is regulated by the planar cell polarity signaling pathway. *Dev Cell* **19**, 138-147.
- CancerResearchUK.** (2012). Latest cancer statistics publications [online]. Available at <http://publications.cancerresearchuk.org/cancerstats/statsskin/keyfactsskin.html> [Accessed on 15 January 2012].
- Chen, M., Marinkovich, M. P., Jones, J. C., O'Toole, E. A., Li, Y. Y. and Woodley, D. T.** (1999). NC1 domain of type VII collagen binds to the beta3 chain of laminin 5 via a unique subdomain within the fibronectin-like repeats. *J Invest Dermatol* **112**, 177-183.
- Chen, M., Marinkovich, M. P., Veis, A., Cai, X., Rao, C. N., O'Toole, E. A. and Woodley, D. T.** (1997). Interactions of the amino-terminal noncollagenous (NC1) domain of type VII collagen with extracellular matrix components. A potential role in epidermal-dermal adherence in human skin. *J Biol Chem* **272**, 14516-14522.
- Christiano, A. M., Greenspan, D. S., Hoffman, G. G., Zhang, X., Tamai, Y., Lin, A. N., Dietz, H. C., Hovnanian, A. and Uitto, J.** (1993). A missense mutation in type VII collagen in two affected siblings with recessive dystrophic epidermolysis bullosa. *Nature Genetics* **4**, 62-66.
- Cox, E. A., Sastry, S. K. and Huttenlocher, A.** (2001). Integrin-mediated adhesion regulates cell polarity and membrane protrusion through the Rho family of GTPases. *Mol Biol Cell* **12**, 265-277.
- Czarnecki, D., Staples, M., Mar, A., Giles, G. and Meehan, C.** (1994). Metastases from squamous cell carcinoma of the skin in southern Australia. *Dermatology* **189**, 52-54.
- Daley, W. P., Gervais, E. M., Centanni, S. W., Gulfo, K. M., Nelson, D. A. and Larsen, M.** (2012). ROCK1-directed basement membrane positioning coordinates epithelial tissue polarity. *Development* **139**, 411-422.
- den Elzen, N., Buttery, C. V., Maddugoda, M. P., Ren, G. and Yap, A. S.** (2009). Cadherin adhesion receptors orient the mitotic spindle during symmetric cell division in mammalian epithelia. *Mol Biol Cell* **20**, 3740-3750.
- Dowling, J., Yu, Q. C. and Fuchs, E.** (1996). Beta4 integrin is required for hemidesmosome formation, cell adhesion and cell survival. *J Cell Biol* **134**, 559-572.
- Ehrenfeld, P., Conejeros, I., Pavicic, M. F., Matus, C. E., Gonzalez, C. B., Quest, A. F., Bhoola, K. D., Poblete, M. T., Burgos, R. A. and Figueroa, C. D.** (2011). Activation of kinin B1 receptor increases the release of metalloproteases-2 and -9 from both estrogen-sensitive and -insensitive breast cancer cells. *Cancer Letters* **301**, 106-118.
- Fears, T. R. and Scotto, J.** (1983). Estimating increases in skin cancer morbidity due to increases in ultraviolet radiation exposure. *Cancer Invest* **1**, 119-126.

- Fine, J. D., Johnson, L. B., Weiner, M., Li, K. P. and Suchindran, C.** (2009). Epidermolysis bullosa and the risk of life-threatening cancers: the National EB Registry experience, 1986-2006. *J Am Acad Dermatol* **60**, 203-211.
- Goldstein, B. and Macara, I. G.** (2007). The PAR proteins: fundamental players in animal cell polarization. *Dev Cell* **13**, 609-622.
- Hagenbuch, B. and Gui, C.** (2008). Xenobiotic transporters of the human organic anion transporting polypeptides (OATP) family. *Xenobiotica* **38**, 778-801.
- Hamada, A., Sissung, T., Price, D. K., Danesi, R., Chau, C. H., Sharifi, N., Venzon, D., Maeda, K., Nagao, K., Sparreboom, A. et al.** (2008). Effect of SLCO1B3 haplotype on testosterone transport and clinical outcome in caucasian patients with androgen-independent prostatic cancer. *Clin Cancer Res* **14**, 3312-3318.
- Hamill, K. J., Hopkinson, S. B., DeBiase, P. and Jones, J. C.** (2009). BPAG1e maintains keratinocyte polarity through beta4 integrin-mediated modulation of Rac1 and cofilin activities. *Mol Biol Cell* **20**, 2954-2962.
- Harwood, C. A. and Proby, C. M.** (2002). Human papillomaviruses and non-melanoma skin cancer. *Curr Opin Infect Dis* **15**, 101-114.
- Ho, E. and Dagnino, L.** (2012a). Emerging role of ILK and ELMO2 in the integration of adhesion and migration pathways. *Cell Adh Migr* **6**, 168-172.
- Ho, E. and Dagnino, L.** (2012b). Epidermal growth factor induction of front-rear polarity and migration in keratinocytes is mediated by integrin-linked kinase and ELMO2. *Mol Biol Cell* **23**, 492-502.
- Ho, E., Irvine, T., Vilks, G. J., Lajoie, G., Ravichandran, K. S., D'Souza, S. J. and Dagnino, L.** (2009). Integrin-linked kinase interactions with ELMO2 modulate cell polarity. *Mol Biol Cell* **20**, 3033-3043.
- Hopkinson, I., Anglin, I. E., Evans, D. L. and Harding, K. G.** (1997). Collagen VII expression in human chronic wounds and scars. *J Pathol* **182**, 192-196.
- Iden, S., van Riel, W. E., Schafer, R., Song, J. Y., Hirose, T., Ohno, S. and Collard, J. G.** (2012). Tumor type-dependent function of the par3 polarity protein in skin tumorigenesis. *Cancer Cell* **22**, 389-403.
- Izu, Y., Sun, M., Zwolanek, D., Veit, G., Williams, V., Cha, B., Jepsen, K. J., Koch, M. and Birk, D. E.** (2011). Type XII collagen regulates osteoblast polarity and communication during bone formation. *J Cell Biol* **193**, 1115-1130.
- Jones, J. C., Kurpakus, M. A., Cooper, H. M. and Quaranta, V.** (1991). A function for the integrin alpha 6 beta 4 in the hemidesmosome. *Cell Regul* **2**, 427-438.
- Jung, D., Podvinec, M., Meyer, U. A., Mangelsdorf, D. J., Fried, M., Meier, P. J. and Kullak-Ublick, G. A.** (2002). Human organic anion transporting polypeptide 8 promoter is transactivated by the farnesoid X receptor/bile acid receptor. *Gastroenterology* **122**, 1954-1966.
- Kim, N. G., Koh, E., Chen, X. and Gumbiner, B. M.** (2011). E-cadherin mediates contact inhibition of proliferation through Hippo signaling-pathway components. *Proc Natl Acad Sci U S A* **108**, 11930-11935.
- Kinsella, T. M. and Nolan, G. P.** (1996). Episomal vectors rapidly and stably produce high-titer recombinant retrovirus. *Hum Gene Ther* **7**, 1405-1413.
- Klaassen, C. D. and Aleksunes, L. M.** (2010). Xenobiotic, bile acid, and cholesterol transporters: function and regulation. *Pharmacol Rev* **62**, 1-96.
- Kligys, K., Claiborne, J. N., DeBiase, P. J., Hopkinson, S. B., Wu, Y., Mizuno, K. and Jones, J. C.** (2007). The slingshot family of phosphatases mediates Rac1 regulation of cofilin phosphorylation, laminin-332 organization, and motility behavior of keratinocytes. *J Biol Chem* **282**, 32520-32528.

- Le Borgne, R., Bellaiche, Y. and Schweisguth, F.** (2002). Drosophila E-cadherin regulates the orientation of asymmetric cell division in the sensory organ lineage. *Curr Biol* **12**, 95-104.
- Lechler, T. and Fuchs, E.** (2005). Asymmetric cell divisions promote stratification and differentiation of mammalian skin. *Nature* **437**, 275-280.
- Li, S., Edgar, D., Fassler, R., Wadsworth, W. and Yurchenco, P. D.** (2003). The role of laminin in embryonic cell polarization and tissue organization. *Dev Cell* **4**, 613-624.
- Lim, S. T., Chen, X. L., Tomar, A., Miller, N. L., Yoo, J. and Schlaepfer, D. D.** (2010). Knock-in mutation reveals an essential role for focal adhesion kinase activity in blood vessel morphogenesis and cell motility-polarity but not cell proliferation. *J Biol Chem* **285**, 21526-21536.
- Lockhart, A. C., Harris, E., Lafleur, B. J., Merchant, N. B., Washington, M. K., Resnick, M. B., Yeatman, T. J. and Lee, W.** (2008). Organic anion transporting polypeptide 1B3 (OATP1B3) is overexpressed in colorectal tumors and is a predictor of clinical outcome. *Clin Exp Gastroenterol* **1**, 1-7.
- Maeda, T., Irokawa, M., Arakawa, H., Kuraoka, E., Nozawa, T., Tateoka, R., Itoh, Y., Nakanishi, T. and Tamai, I.** (2010). Uptake transporter organic anion transporting polypeptide 1B3 contributes to the growth of estrogen-dependent breast cancer. *J Steroid Biochem Mol Biol* **122**, 180-185.
- Mallipeddi, R., Wessagowit, V., South, A. P., Robson, A. M., Orchard, G. E., Eady, R. A. and McGrath, J. A.** (2004). Reduced expression of insulin-like growth factor-binding protein-3 (IGFBP-3) in Squamous cell carcinoma complicating recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* **122**, 1302-1309.
- Martin-Belmonte, F. and Mostov, K.** (2008). Regulation of cell polarity during epithelial morphogenesis. *Curr Opin Cell Biol* **20**, 227-234.
- Martin-Belmonte, F. and Perez-Moreno, M.** (2012). Epithelial cell polarity, stem cells and cancer. *Nat Rev Cancer* **12**, 23-38.
- Martin-Belmonte, F., Yu, W., Rodriguez-Fraticelli, A. E., Ewald, A. J., Werb, Z., Alonso, M. A. and Mostov, K.** (2008). Cell-polarity dynamics controls the mechanism of lumen formation in epithelial morphogenesis. *Curr Biol* **18**, 507-513.
- Martins, V. L., Vyas, J. J., Chen, M., Purdie, K., Mein, C. A., South, A. P., Storey, A., McGrath, J. A. and O'Toole, E. A.** (2009). Increased invasive behaviour in cutaneous squamous cell carcinoma with loss of basement-membrane type VII collagen. *J Cell Sci* **122**, 1788-1799.
- McGrath, J. A., Leigh, I. M. and Eady, R. A.** (1992). Intracellular expression of type VII collagen during wound healing in severe recessive dystrophic epidermolysis bullosa and normal human skin. *Br J Dermatol* **127**, 312-317.
- McGregor, J. M. and Proby, C. M.** (1996). The role of papillomaviruses in human non-melanoma skin cancer. *Cancer Surv* **26**, 219-236.
- Miller, D. L. and Weinstock, M. A.** (1994). Nonmelanoma skin cancer in the United States: incidence. *J Am Acad Dermatol* **30**, 774-778.
- Munro, E. M.** (2006). PAR proteins and the cytoskeleton: a marriage of equals. *Curr Opin Cell Biol* **18**, 86-94.
- Muroyama, A. and Lechler, T.** (2012). Polarity and stratification of the epidermis. *Semin Cell Dev Biol* **23**, 890-896.
- Nagai, M., Furihata, T., Matsumoto, S., Ishii, S., Motohashi, S., Yoshino, I., Ugajin, M., Miyajima, A. and Chiba, K.** (2012). Identification of a new organic anion transporting polypeptide 1B3 mRNA isoform primarily expressed in human cancerous tissues and cells. *Biochem Biophys Res Commun* **418**, 818-823.

Navarro, C., Nola, S., Audebert, S., Santoni, M. J., Arsanto, J. P., Ginestier, C., Marchetto, S., Jacquemier, J., Isnardon, D., Le Bivic, A. et al. (2005). Junctional recruitment of mammalian Scribble relies on E-cadherin engagement. *Oncogene* **24**, 4330-4339.

Ng, Y.-Z., Dayal, J. H. S. and South, A. P. (2011). Genetic Predisposition to Cutaneous Squamous Cell Carcinoma. *Skin Cancers - Risk Factors, Prevention and Therapy, Caterina AM La Porta (Ed.)*, ISBN: 978-953-307-722-972, InTech, Available from: <http://www.intechopen.com/books/skin-cancers-risk-factors-prevention-and-therapy/genetic-predisposition-to-cutaneous-squamous-cell-carcinoma>

Ng, Y. Z., Pourreyron, C., Salas-Alanis, J. C., Dayal, J. H., Cepeda-Valdes, R., Yan, W., Wright, S., Chen, M., Fine, J. D., Hogg, F. J. et al. (2012). Fibroblast-derived dermal matrix drives development of aggressive cutaneous squamous cell carcinoma complicating patients with recessive dystrophic epidermolysis bullosa. *Cancer Research*.

Niessen, M. T., Iden, S. and Niessen, C. M. (2012). The in vivo function of mammalian cell and tissue polarity regulators--how to shape and maintain the epidermal barrier. *J Cell Sci* **125**, 3501-3510.

Nony, P. A. and Schnellmann, R. G. (2001). Interactions between collagen IV and collagen-binding integrins in renal cell repair after sublethal injury. *Mol Pharmacol* **60**, 1226-1234.

Nystrom, M. L., Thomas, G. J., Stone, M., Mackenzie, I. C., Hart, I. R. and Marshall, J. F. (2005). Development of a quantitative method to analyse tumour cell invasion in organotypic culture. *J Pathol* **205**, 468-475.

Obaidat, A., Roth, M. and Hagenbuch, B. (2012). The expression and function of organic anion transporting polypeptides in normal tissues and in cancer. *Annu Rev Pharmacol Toxicol* **52**, 135-151.

Ogawa, T., Tsubota, Y., Hashimoto, J., Kariya, Y. and Miyazaki, K. (2007). The short arm of laminin gamma2 chain of laminin-5 (laminin-332) binds syndecan-1 and regulates cellular adhesion and migration by suppressing phosphorylation of integrin beta4 chain. *Mol Biol Cell* **18**, 1621-1633.

Olk, S., Zoidl, G. and Dermietzel, R. (2009). Connexins, cell motility, and the cytoskeleton. *Cell Motil Cytoskeleton* **66**, 1000-1016.

Plachot, C., Chaboub, L. S., Adissu, H. A., Wang, L., Urazaev, A., Sturgis, J., Asem, E. K. and Lelievre, S. A. (2009). Factors necessary to produce basoapical polarity in human glandular epithelium formed in conventional and high-throughput three-dimensional culture: example of the breast epithelium. *BMC Biol* **7**, 77.

Poulson, N. D. and Lechler, T. (2010). Robust control of mitotic spindle orientation in the developing epidermis. *J Cell Biol* **191**, 915-922.

Pressler, H., Sissung, T. M., Venzon, D., Price, D. K. and Figg, W. D. (2011). Expression of OATP family members in hormone-related cancers: potential markers of progression. *PLoS One* **6**, e20372.

Preston, D. S. and Stern, R. S. (1992). Nonmelanoma cancers of the skin. *N Engl J Med* **327**, 1649-1662.

Ramachandran, A., Betts, G., Bhana, S., Helme, G., Blick, C., Moller-Levet, C., Saunders, E., Valentine, H., Pepper, S., Miller, C. J. et al. (2013). An in vivo hypoxia metagene identifies the novel hypoxia inducible factor target gene SLC01B3. *Eur J Cancer* **49**, 1741-1751.

Reed, W. B., Roenigk, H., Jr., Dorner, W., Jr., Welsh, O. and Martin, F. J. (1975). Epidermal neoplasms with epidermolysis bullosa dystrophica with the first report of carcinoma with the acquired type. *Arch Dermatol Res* **253**, 1-14.

- Rousselle, P., Keene, D. R., Ruggiero, F., Champlaud, M. F., Rest, M. and Burgeson, R. E.** (1997). Laminin 5 binds the NC-1 domain of type VII collagen. *J Cell Biol* **138**, 719-728.
- Rowe, D. E., Carroll, R. J. and Day, C. L., Jr.** (1992). Prognostic factors for local recurrence, metastasis, and survival rates in squamous cell carcinoma of the skin, ear, and lip. Implications for treatment modality selection. *J Am Acad Dermatol* **26**, 976-990.
- Sakai, L. Y., Keene, D. R., Morris, N. P. and Burgeson, R. E.** (1986). Type VII collagen is a major structural component of anchoring fibrils. *J Cell Biol* **103**, 1577-1586.
- Smith, N. F., Figg, W. D. and Sparreboom, A.** (2005a). Role of the liver-specific transporters OATP1B1 and OATP1B3 in governing drug elimination. *Expert Opin Drug Metab Toxicol* **1**, 429-445.
- Smith, N. F., Acharya, M. R., Desai, N., Figg, W. D. and Sparreboom, A.** (2005b). Identification of OATP1B3 as a high-affinity hepatocellular transporter of paclitaxel. *Cancer Biol Ther* **4**, 815-818.
- South, A. P. and O'Toole, E. A.** (2010). Understanding the pathogenesis of recessive dystrophic epidermolysis bullosa squamous cell carcinoma. *Dermatol Clin* **28**, 171-178.
- Svoboda, M., Wlcek, K., Taferner, B., Hering, S., Stieger, B., Tong, D., Zeillinger, R., Thalhammer, T. and Jager, W.** (2011). Expression of organic anion-transporting polypeptides 1B1 and 1B3 in ovarian cancer cells: relevance for paclitaxel transport. *Biomed Pharmacother* **65**, 417-426.
- Thakkar, N., Kim, K., Jang, E. R., Han, S., Kim, D., Merchant, N., Lockhart, A. C. and Lee, W.** (2013). A Cancer-Specific Variant of the SLCO1B3 Gene Encodes a Novel Human Organic Anion Transporting Polypeptide 1B3 (OATP1B3) Localized Mainly in the Cytoplasm of Colon and Pancreatic Cancer Cells. *Mol Pharm* **10**, 406-416.
- Toms, J.** (2004). CancerStats Monograph. London: Cancer Research UK.
- Ulvestad, M., Bjorquist, P., Molden, E., Asberg, A. and Andersson, T. B.** (2011). OATP1B1/1B3 activity in plated primary human hepatocytes over time in culture. *Biochem Pharmacol* **82**, 1219-1226.
- Veness, M. J., Quinn, D. I., Ong, C. S., Keogh, A. M., Macdonald, P. S., Cooper, S. G. and Morgan, G. W.** (1999). Aggressive cutaneous malignancies following cardiothoracic transplantation: the Australian experience. *Cancer* **85**, 1758-1764.
- Villone, D., Fritsch, A., Koch, M., Bruckner-Tuderman, L., Hansen, U. and Bruckner, P.** (2008). Supramolecular interactions in the dermo-epidermal junction zone: anchoring fibril-collagen VII tightly binds to banded collagen fibrils. *J Biol Chem* **283**, 24506-24513.
- Wang, T. W., Sun, J. S., Huang, Y. C., Wu, H. C., Chen, L. T. and Lin, F. H.** (2006). Skin basement membrane and extracellular matrix proteins characterization and quantification by real time RT-PCR. *Biomaterials* **27**, 5059-5068.
- Wang, Y.** (2009). Wnt/Planar cell polarity signaling: a new paradigm for cancer therapy. *Mol Cancer Ther* **8**, 2103-2109.
- Watt, S. A., Pourreyron, C., Purdie, K., Hogan, C., Cole, C. L., Foster, N., Pratt, N., Bourdon, J. C., Appleyard, V., Murray, K. et al.** (2011). Integrative mRNA profiling comparing cultured primary cells with clinical samples reveals PLK1 and C20orf20 as therapeutic targets in cutaneous squamous cell carcinoma. *Oncogene* **30**, 4666-4677.
- Weaver, V. M., Petersen, O. W., Wang, F., Larabell, C. A., Briand, P., Damsky, C. and Bissell, M. J.** (1997). Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. *J Cell Biol* **137**, 231-245.
- Weinstock, M. A., Bogaars, H. A., Ashley, M., Little, V., Bilodeau, E. and Kimmell, S.** (1991). Nonmelanoma skin cancer mortality. A population-based study. *Arch Dermatol* **127**, 1194-1197.
- Yanofsky, V. R., Mercer, S. E. and Phelps, R. G.** (2011). Histopathological variants of cutaneous squamous cell carcinoma: a review. *J Skin Cancer*, 210813.

FIGURE LEGENDS

Figure 1: *SLCO1B3* is up-regulated in cSCC derived keratinocytes and primary tissue.

A. *SLCO1B3* mRNA expression as assessed by Sybr®Green qPCR in 6 cSCC, 3 RDEB cSCC, 2 metastatic cSCC, 1 normal human keratinocyte (NHK) and 1 hepatocellular carcinoma (HepG2) culture. The expression levels were calculated using the $\Delta\Delta C_T$ method with *ACTB* as the normaliser and NHK as the calibrator. **B.** Sybr®Green qPCR analysis of *SLCO1B3* mRNA levels in tissue from 11 RDEB cSCC, 3 RDEB skin, 3 cSCC, 1 normal skin (NHS) and 1 normal liver. The expression levels were calculated using the $\Delta\Delta C_T$ method with *ACTB* as the normaliser and NHS as the calibrator. Experiment repeated twice, data from the same experiment, performed in triplicate, is shown as the mean \pm s.d.

Figure 2: OATP1B3 is endogenously expressed in cSCC derived keratinocytes

A. Endogenous OATP1B3 protein expression was detected using immunoblotting in cSCC keratinocytes, but not in normal human keratinocytes (NHK). *SLCO1B3* overexpressing cells (OATP1B3_SCCRDEB2) were used as a positive control. EV_SCCRDEB2 = empty vector control cells. Adjacent western blot quantification graph shows percentage average relative OATP1B3 protein density (n=3, mean \pm s.d). **B.** OATP1B3 protein expression is readily detected in monolayer 2D cultured cells (SCCT8) (left panels) but is only localised to the plasma membrane when it is over-expressed in 2D culture (middle panels) or in mouse xenograft tumours (right panels) using the same SCCT8 cells. Top panel shows immunostaining with an antibody raised against OATP1B3 while bottom panel shows DAPI nuclear staining, Bar = 10 μ m.

Figure 3: OATP1B3 is overexpressed in cSCC, RDEB cSCC and RDEB patient skin but is absent from normal skin.

A. cSCC tissues and RDEB patient skin analysed for OATP1B3 showed a strong plasma membrane protein expression along with nuclear localisation. Normal liver was used as a positive control and normal skin as a negative control for immunofluorescence. Top panel shows OATP1B3 immunostaining and the lower panel shows DAPI counter stain for all tissues. Bar = 10 μ m. **B.** Confocal images of cSCC tissue showing OATP1B3 in red and

plakoglobin in green. The merge image shows OATP1B3 nuclear staining (indicated by yellow arrows) and plasma membrane co-localisation with plakoglobin (indicated by white arrows). Bar = 10 μ m.

Figure 4: *SLCO1B3* expression is negatively regulated by full length type VII collagen in both RDEB cSCC and UV induced cSCC keratinocytes.

A. SCCRDEB3 keratinocytes transduced with full length *COL7A1* (clones C7-6 and C7-9) show reduced *SLCO1B3* mRNA levels compared to the empty vector cells (EV) as determined by Sybr®Green qPCR analysis. The expression levels were calculated using the $\Delta\Delta$ CT method with *ACTB* as the normaliser and EV as the calibrator. **B.** Western blot confirms type VII collagen overexpression and reduced OATP1B3 expression in both SCCRDEB3 C7-6 and C7-9 clones compared to the empty vector control. **C.** Western blot quantification shows relative average OATP1B3 protein density from **B** (n=3, mean \pm s.d.). **D.** A dual luciferase gene reporter assay was designed to assess the effect of *COL7A1* on *SLCO1B3* promoter activity, which was significantly reduced in the type VII collagen overexpressing cells (C7-6), compared to the empty vector (EV) controls. **E.** *SLCO1B3* expression is significantly reduced when SCCRDEB3 and SCCRDEB2 cells are cultured on matrix derived from type VII collagen overexpressing cells (C7-6) compared to the empty vector control (EV). Experiments performed in triplicate and data is shown as the means \pm s.d. **p<0.005 and *p<0.05.

Figure 5: *SLCO1B3* mRNA expression is increased following *COL7A1* knock down in both UV induced and RDEB cSCC keratinocytes.

A. Type VII collagen knock down was achieved using three siRNA constructs in clonal populations of SCCRDEB3 C7 and EV cells (SiC7-1, SiC7-2 and SiC7-3) and compared to the non-targeting control (NT). **B.** *SLCO1B3* mRNA levels showed a significant increase in type VII collagen overexpressing cells following *COL7A1* knock down compared to the empty vector control cells. The same assay was repeated for **C.** SCCRDEB4-C7 and **D.** SCCT8 cells.

Figure 6: Type VII collagen positively regulates ELMO2 expression in cSCC keratinocytes.

A. Type VII collagen re-expression in SCCRDEB3 keratinocytes (C7-6) results in an increase in *ELMO2* expression. The expression levels were calculated using the $\Delta\Delta CT$ method with *GAPDH* as the normaliser and empty vector control (EV) as the calibrator. **B.** Type VII collagen knock down was achieved using a siRNA pool (SiC7 pool) and compared to non-targeting control (NT). *ELMO2* mRNA levels showed a significant decrease in both UV induced cSCC and type VII collagen overexpressing RDEB cSCC keratinocytes following *COL7A1* knock down compared to the empty vector control cells. **C.** Western blot confirms an increase in *ELMO2* expression in SCCRDEB3 keratinocytes following *COL7A1* expression and a reduction in *ELMO2* expression in UV induced cSCC keratinocytes (SCCT1) following *COL7A1* knock down. Adjacent western blot quantification graphs show percentage average relative *ELMO2* protein density (n=3, mean \pm s.d). **D.** Immunofluorescence images confirm the loss of *ELMO2* expression following type VII collagen knock down in SCCT8. Bar = 10 μ m **E.** *ELMO2* expression was significantly increased when SCCRDEB3 and SCCRDEB2 cells were cultured on matrix derived from type VII collagen overexpressing cells (C7-6) compared to the empty vector control (EV). **F.** Immunofluorescence images confirming an increase in *ELMO2* expression when SCCRDEB3 cells were cultured on matrix derived from C7-6 cells compared to EV. Bar = 10 μ m. Experiments performed in triplicate and the data is shown as the means \pm s.d. **p<0.005 and *p<0.05.

Figure 7: *ELMO2* and Ln332 regulate *SLCO1B3* expression in cSCC keratinocytes

A. *ELMO2* was transiently overexpressed in SCCT8 and SCCRDEB2 cells. Inset western blot confirms *ELMO2* overexpression in SCCRDEB2 cells. *ELMO2* = overexpressing cells and EV = empty vector control **B.** *SLCO1B3* expression was reduced in both SCCT8 and SCCRDEB2 keratinocytes following *ELMO2* overexpression, **C.** *ELMO2* knock down was achieved in SCCT8 using a pool of three siRNA constructs (SiEL2 pool). **D.** *SLCO1B3* mRNA levels were increased following *ELMO2* depletion compared to the non-targeting control (NT). **E.** Western blot showing a reduction of the gamma 2 chain of Ln332 expression in SCCRDEB3 cells following knock down using a pool of three siRNA constructs after 48 and 72 hours. *SLCO1B3* expression was increased in Ln332 knock down cells (SiLn332-48 hr/72 hr) compared to the non-targeting control (NT). Experiment performed in triplicate and represented as the means \pm s.d. *p<0.05.

Figure 8: Type VII collagen overexpression imparts front-to-rear polarity and increased expression of polarity associated proteins in cSCC.

A. Confocal images of individual cells labelled with actin (green) showing retrovirally transduced type VII collagen overexpressing keratinocytes (C7-6) form lamellipodia (indicated by white arrows) and establish front-to-rear polarity earlier in comparison to control cells (EV). Bar = 10 μ m. **B-C.** The adjacent graphs show the percentage of spread cells and lamellipodia formation per spread cell in C7-6 and EV. Experiment performed in triplicate and is represented as the \pm SEM. * $p < 0.005$. **D.** C7-6 3D mono-cultured spheroids show increased structural organisation compared to EV cells. PAR3 expression at the plasma membrane is reduced in EV spheroids compared to C7-6 spheroids. Bar = 10 μ m **E.** Western blot analysis from lysates extracted from C7-6 spheroids show increased Ln332, PAR3, ITAG6 and ELMO2 protein expression compared to EV spheroids. Adjacent western blot quantification graph gives the percentage average relative density for the proteins shown, (n=3, mean \pm s.d). **F.** Type VII collagen overexpressing fibroblasts (C7) impart increased structural organisation when co-cultured with SCCRDEB3 keratinocytes compared to EV fibroblast. 3D culture, H&E and DAPI images show the difference in structural organisation between C7 and EV cells. E-cadherin (green) and β -catenin (red) show reduced plasma membrane localisation and expression in EV cells compared to C7 cells. The adjacent bar graphs show image quantification comparing fluorescence levels for b-catenin and e-cadherin in EV and C7 co-culture spheroids. **G.** western blot showing e-cadherin and beta-catenin protein expression in RDEBSCC3 co-culture spheroids shown in Fig 8F. Bar = 10 μ m.

Figure 1

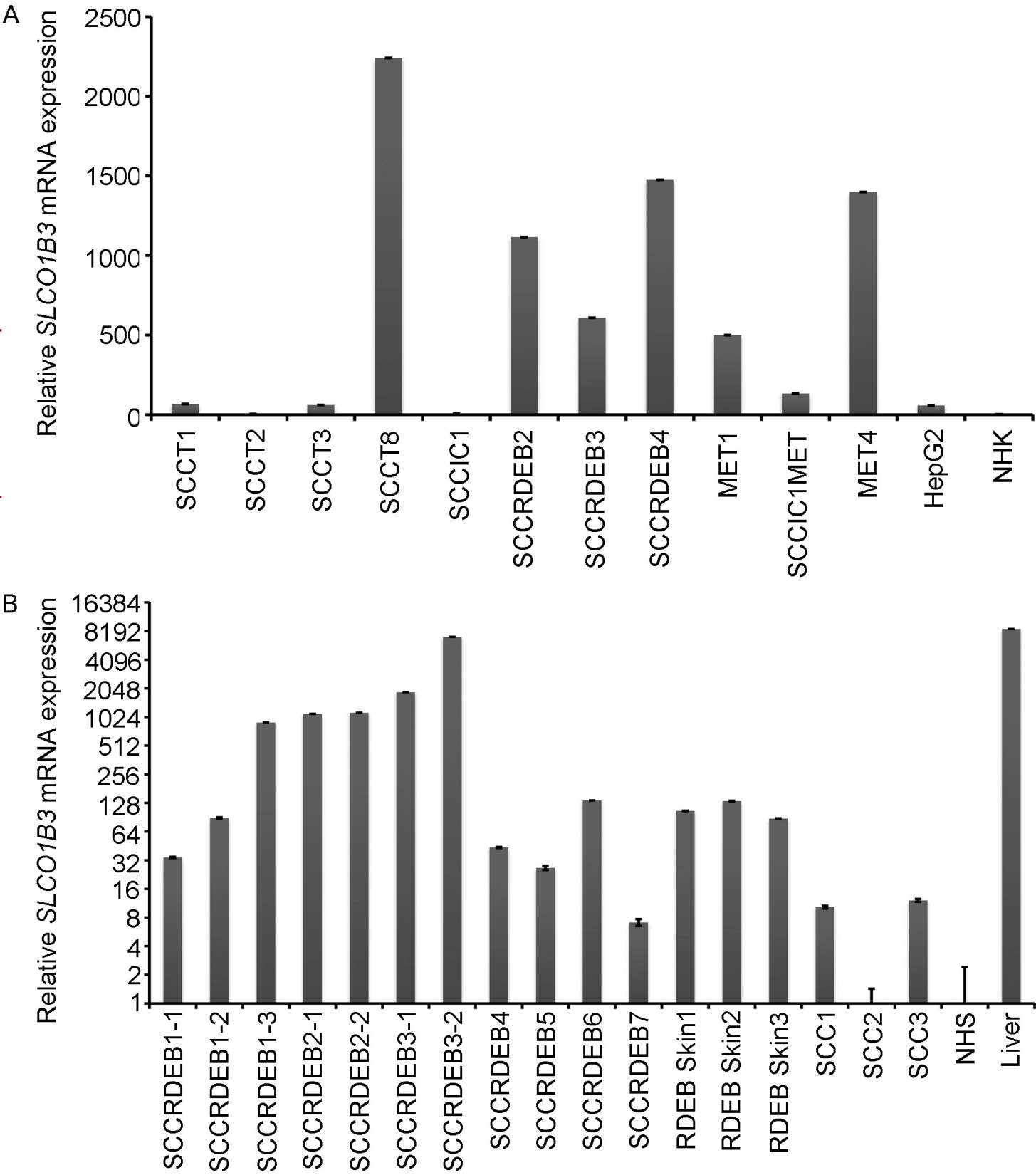


Figure 2

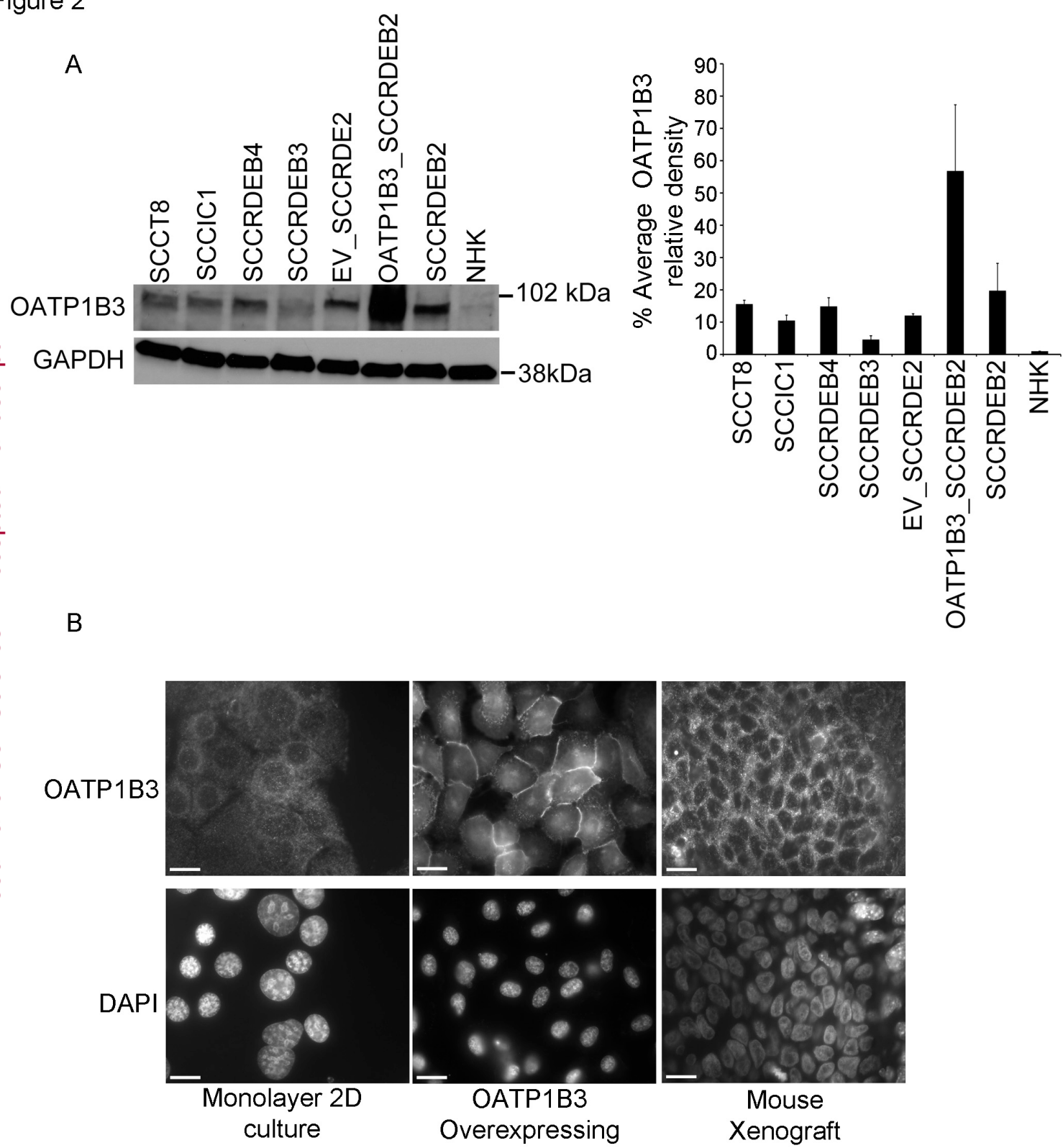


Figure 3

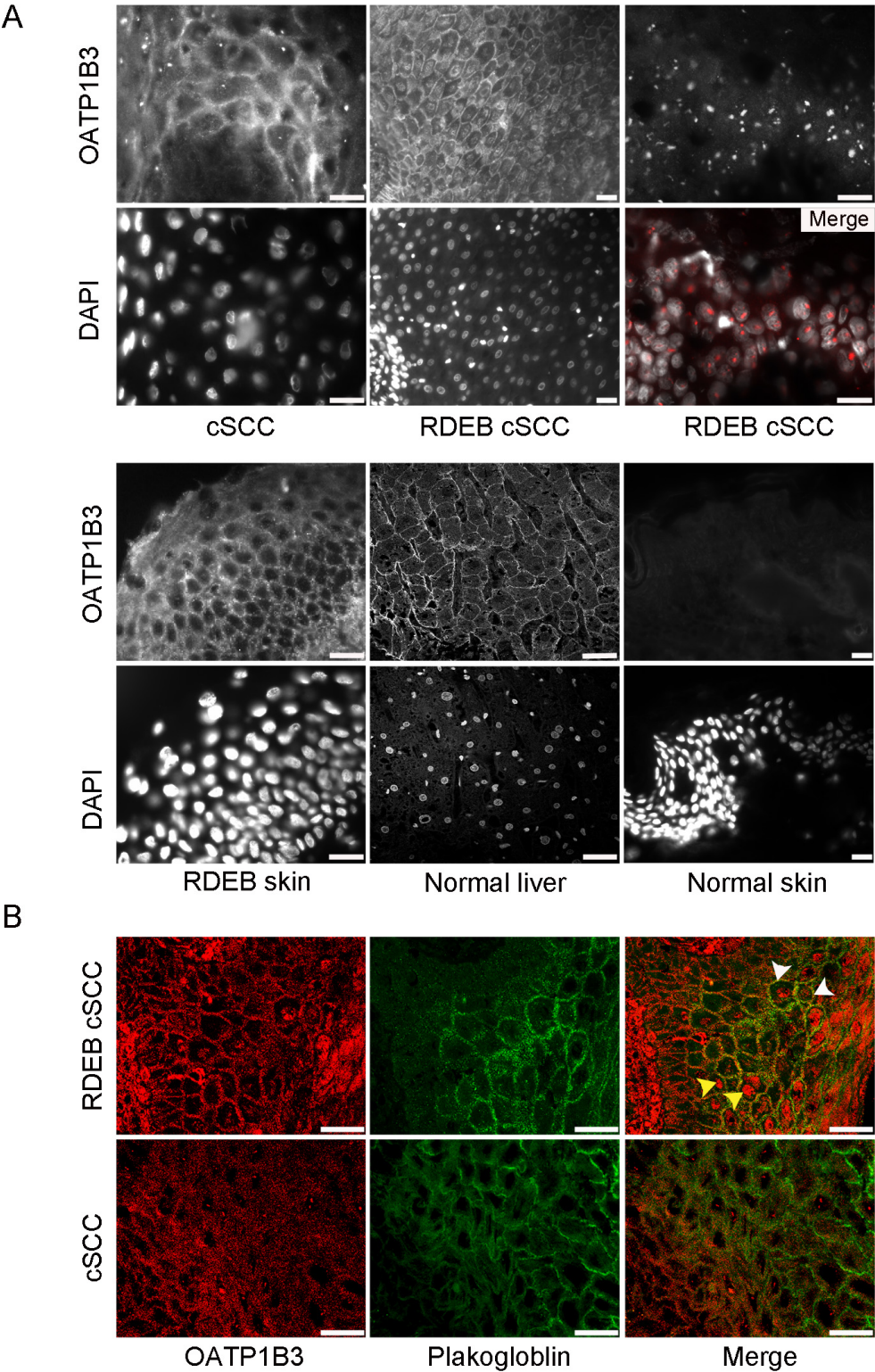
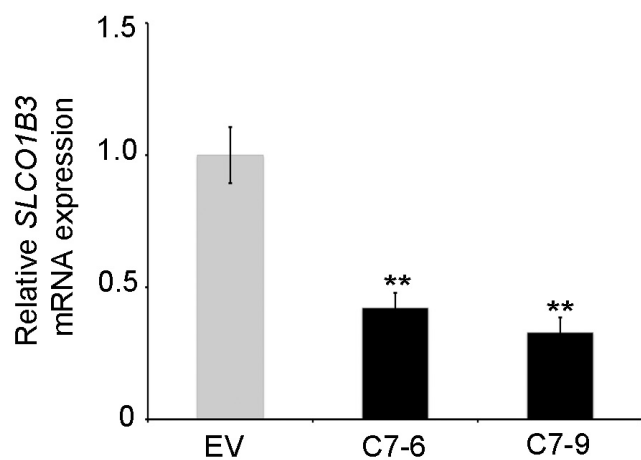
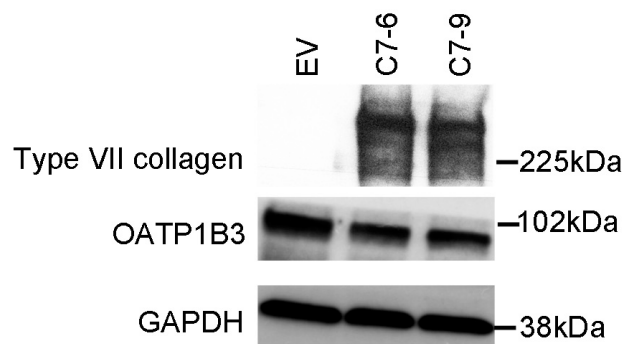


Figure 4

A



B



D

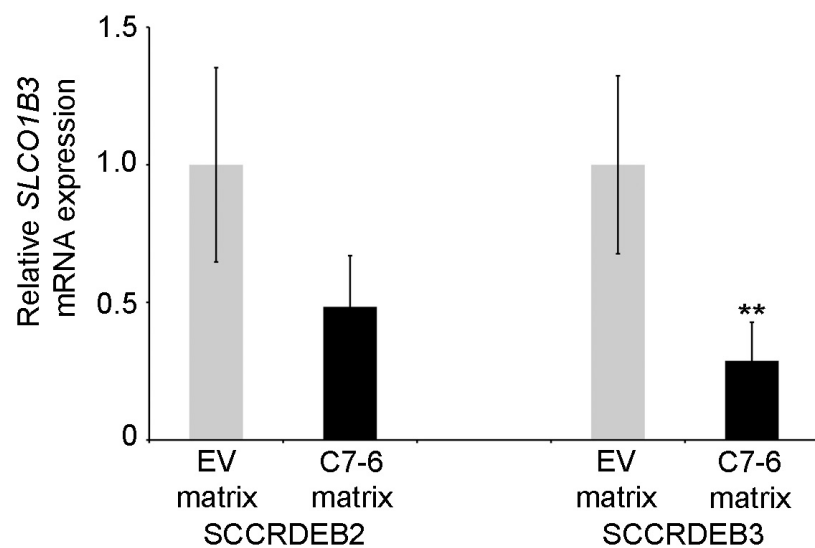
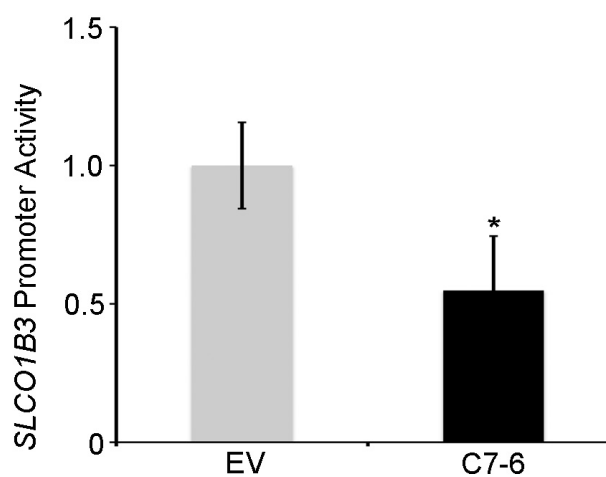


Figure 5

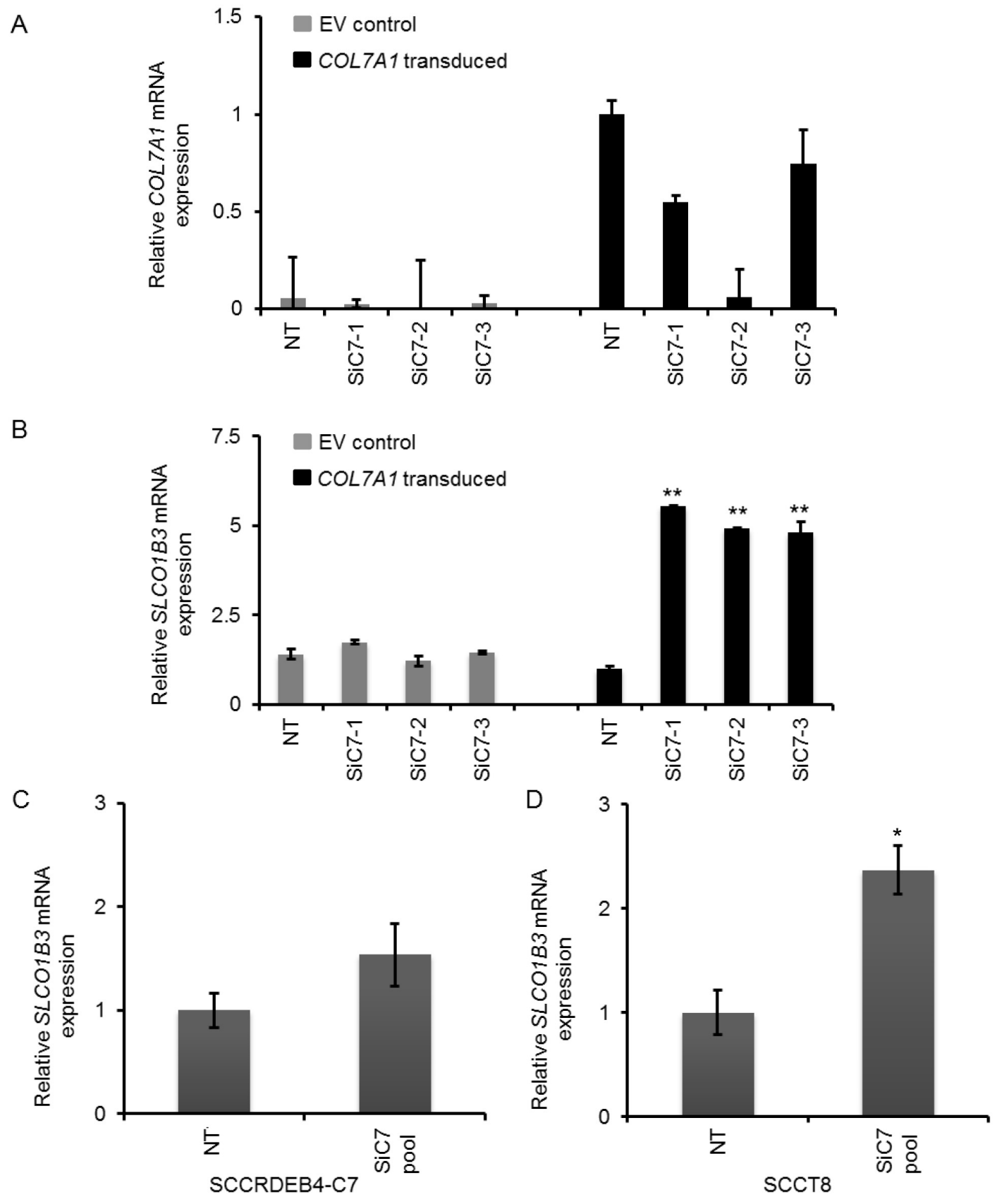


Figure 6

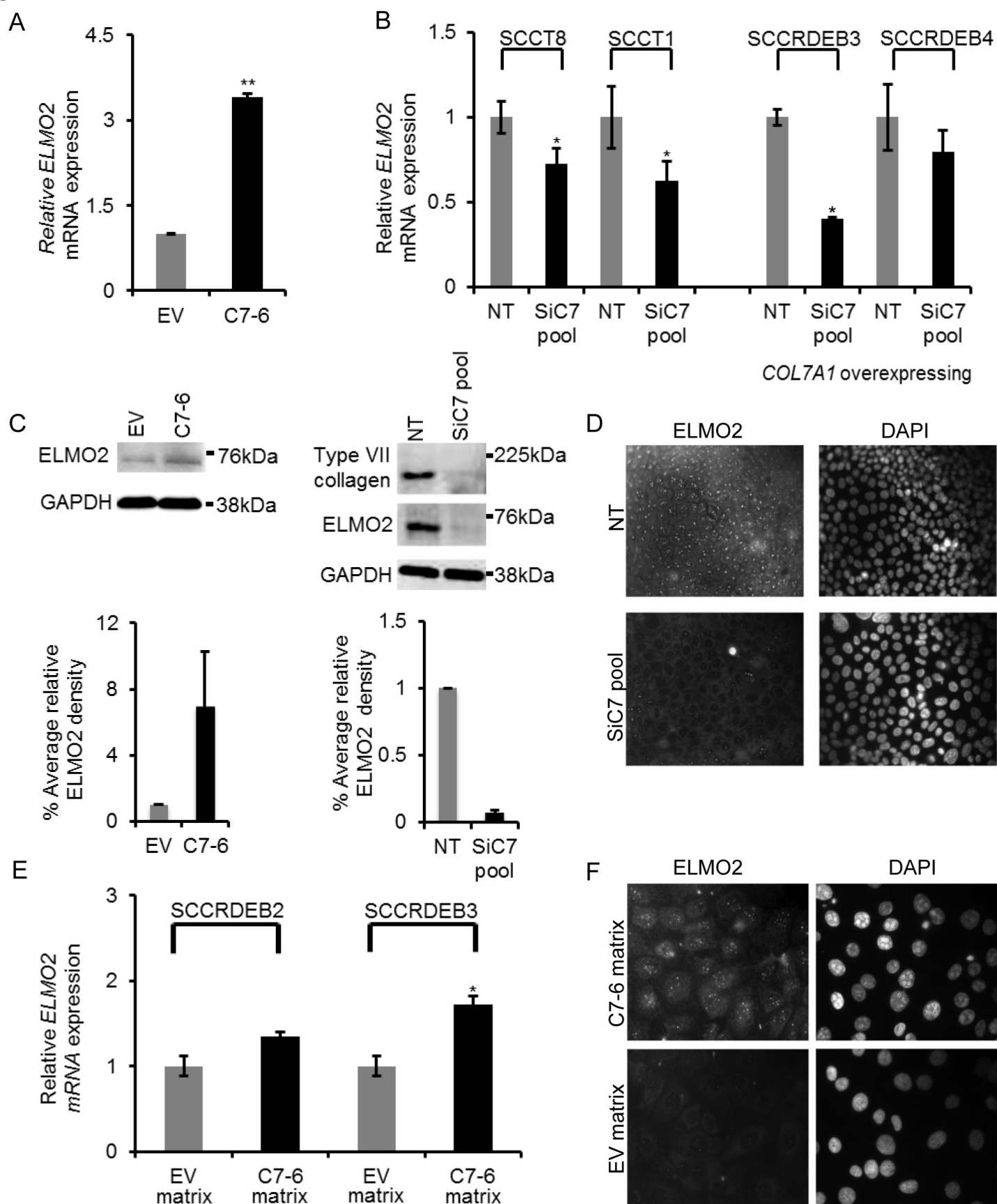


Figure 7

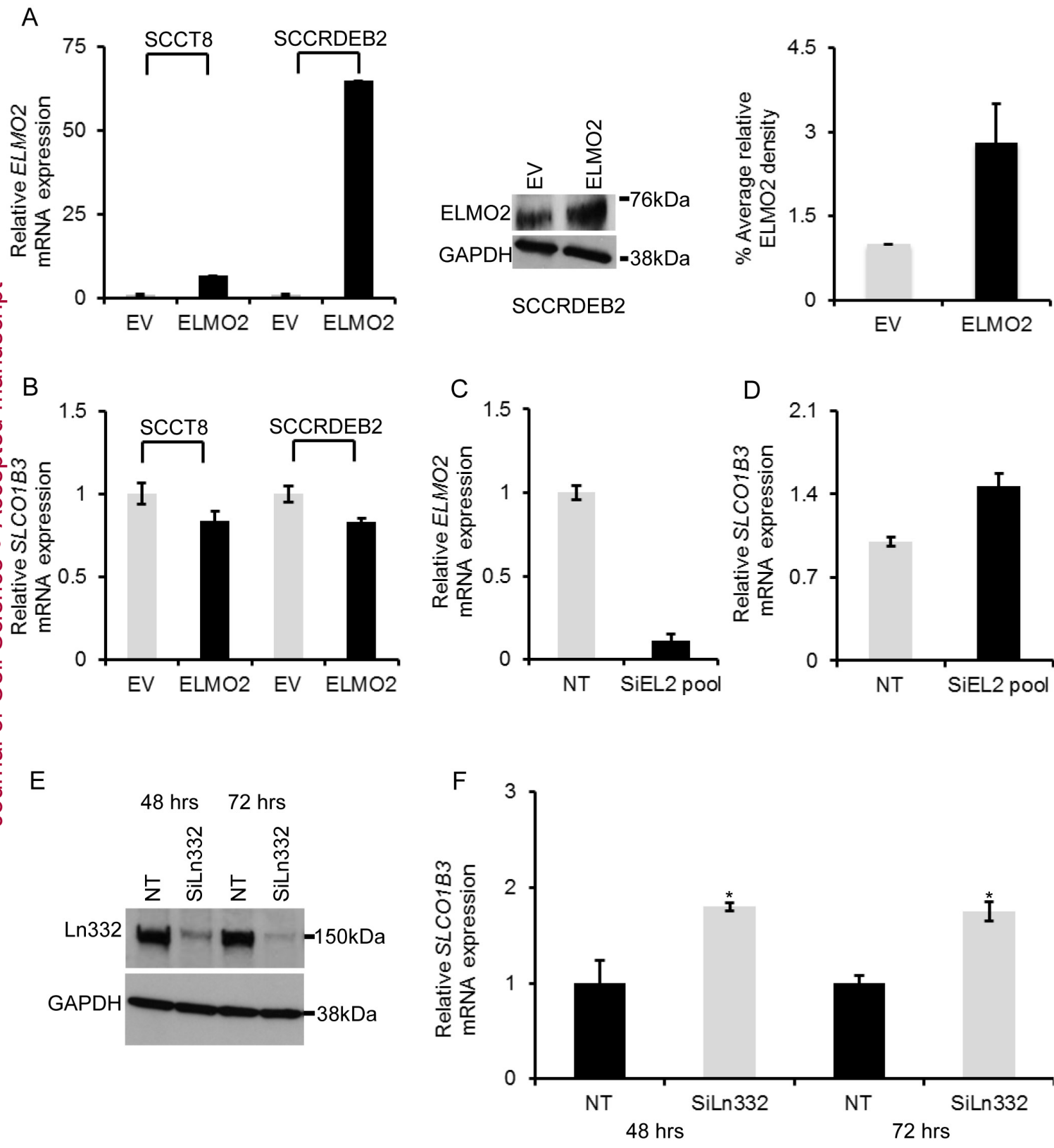


Figure 8

